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Structure-Activity Dependency of New Bacterial Tryptophanyl tRNA Synthetase Inhibitors

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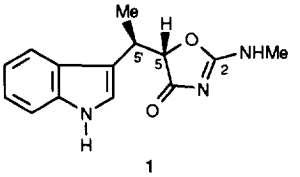
Abstract: Analogues of the aminoacyl tRNA synthetase inhibitor, indolmycin, have been synthesised in which the side chain methyl group is replaced by a wide range of substituents. Their antibacterial and enzyme inhibitory potency is related to steric properties and conformational preferences. Copyright © 1996 Elsevier Science Ltd

The pivotal metabolic role played by bacterial aminoacyl tRNA synthetase enzymes makes them potential targets for antimicrobial strategies.[1] Compounds which can selectively inhibit specific bacterial aminoacyl tRNA synthetases, without affecting their mammalian counterparts, are therefore candidates for therapeutic antibiotics.[2] This has been an area of intense interest and several antibacterial aminoacyl tRNA synthetase inhibitors have been reported, including the natural product indolmycin **1,** a potent and selective competitive inhibitor of the bacterial tryptophanyl enzyme.[2,3] It has also been demonstrated that indolmycin can compete with L-tryptophan **2** in the bacterial amino acid uptake mechanism.[3] While the two molecules have obvious structural similarities, in indolmycin the α-amino acid functionality is replaced by an uncharged 2-methylaminooxazolin-4-one ring, with a 5'-methyl substituent corresponding to the amino acid β-position. Several studies have reported variants of this inhibitor, produced by either substitution on the oxazolinone ring or directed biosynthesis.[4,5] In this communication, the effect of modifying the methyl substituent at the carbon atom bridging the two heterocyclic rings is reported.



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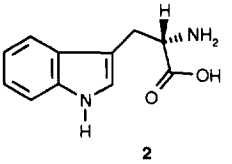

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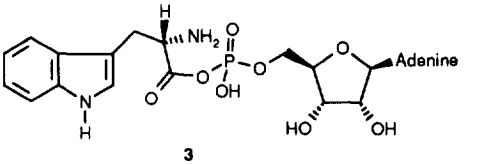

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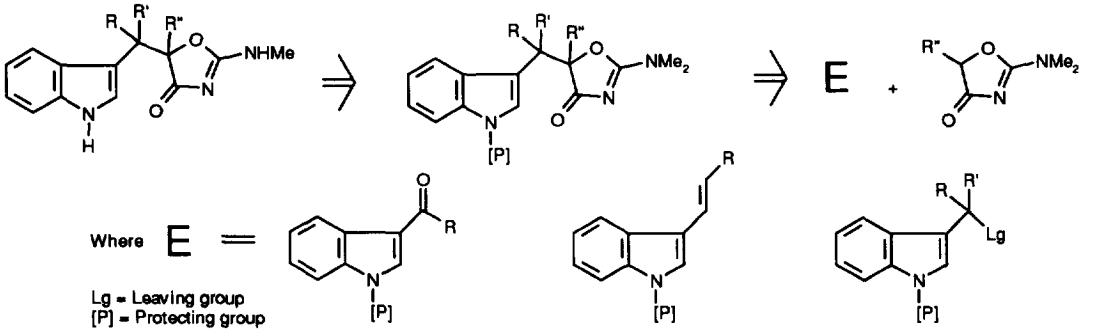
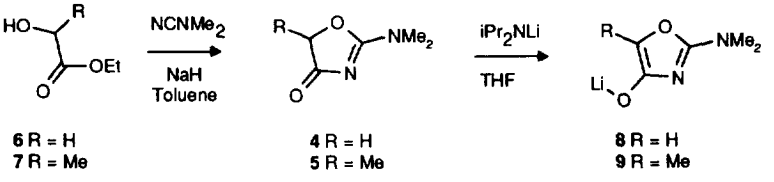
Indolmycin is known to compete for the tryptophan binding site in the synthetase-catalysed process in which tryptophan reacts with ATP to form tryptophanyl adenylate **3**.[6] The methyl group on the carbon atom separating the two rings could exert an effect in several ways: stabilising the particular indolmycin conformation which binds to the enzyme; interacting with a specific enzyme binding site; or it may be aligned with the bound amino acid backbone of tryptophan. In this last case, the oxazolinone moiety does not mimic the amino or carboxylic acid groups. To test these theories, analogues were synthesised in which the 5' substituent was replaced by larger or smaller groups, and by polar species including amino and carboxylic acid functionalities. Adjacent steric crowding is also introduced. The general strategy for their formation is shown in the retrosynthetic analysis of **Scheme 1**.



Scheme 1

The indolmycin analogues were prepared by the condensation of the lithium enolate of an oxazolinone with a suitably protected indole electrophile. This strategy was used by Dirlam and co-workers in their synthesis of indolmycin; they demonstrated that 2-dimethylaminooxazolin-4-one so introduced could be transaminated to the bioactive 2-methylamino compound using methylamine.[5] The 5-H and the 5-methyl oxazolinones **4** and **5** were synthesised (**Scheme 2**) by the reaction of dimethylcyanamide with either ethyl glycolate **6** or ethyl lactate **7** in the presence of base. This process afforded the corresponding crystalline oxazolinones in 55-65% yield. Deprotonation with lithium diisopropylamide furnished the appropriate oxazole lithium enolates **8** and **9** respectively.



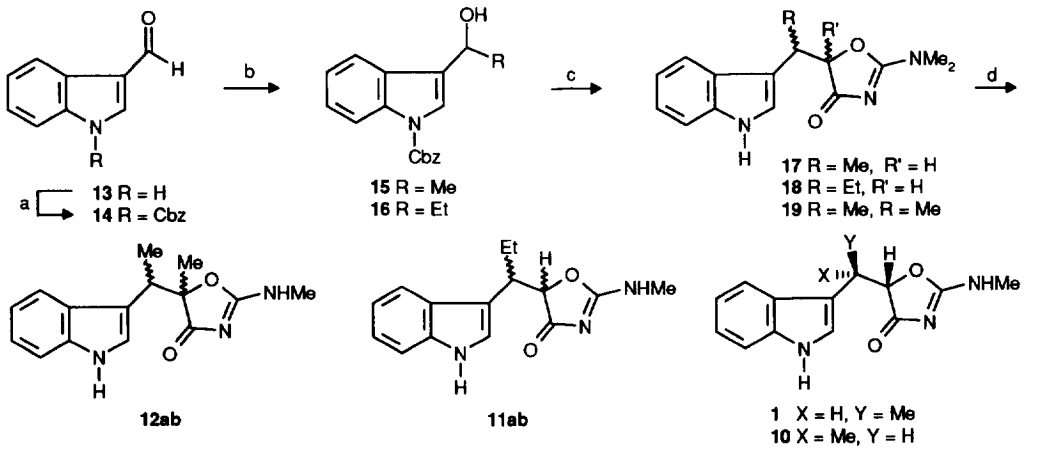
6 R = H
7 R = Me

4 R = H
5 R = Me

8 R = H
9 R = Me

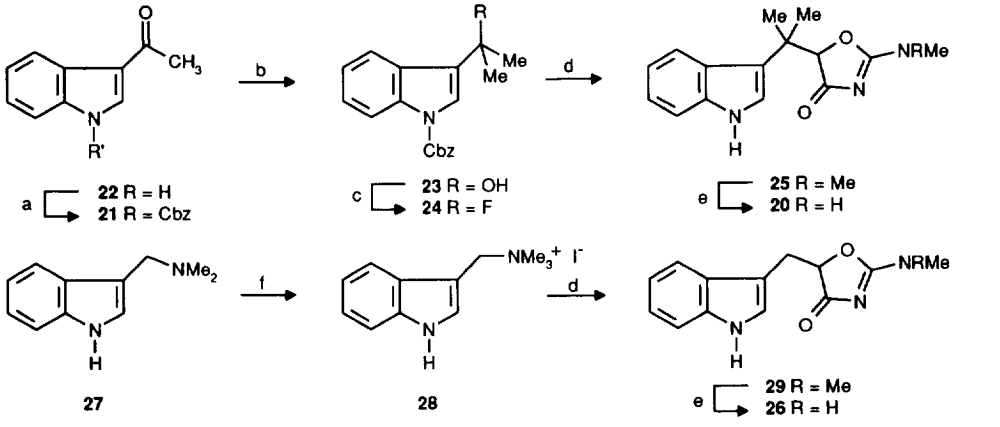
Scheme 2

In the first instance, a range of alkyl substituted analogues was prepared. All of the compounds described were synthesised as racemates; a comparison of the racemic and resolved enantiomers of indolmycin indicated that essentially all the enzyme inhibitory and antibacterial properties resided in the 5(S), 5'(R)-isomer **1**. Both indolmycin diastereomers **1** and **10**, its ethyl counterpart **11ab** and the 5-methyloxazolinone **12ab** were prepared by variants of the same route (**Scheme 3**). Indole-3-carboxaldehyde **13** was N-protected with a Cbz group to give **14** then treated with one equivalent of the methyl or ethyl Grignard reagent to afford the corresponding alcohols **15** and **16** in 72% and 79% overall yield respectively. These reacted with thionyl chloride to give intermediate chlorides, which on treatment with the oxazolinone lithium enolates **8** and **9** formed the corresponding diastereomeric adducts **17**, **18** and **19** in overall yields of between 25% and 70%. The diastereomers were separated and each transaminated without racemisation by exposure to methylamine to afford respectively indolmycin **1**, isoindolmycin **10** and both diastereomers of the two analogues **11ab** and **12ab**, in yields of between 25% and 80%.



Scheme 3 a) NaH, THF, then $PhCH_2OCOCl$; b) RMgBr, THF, c) $SOCl_2$, then **8** or **9** / THF; d) NH_2Me, sealed tube

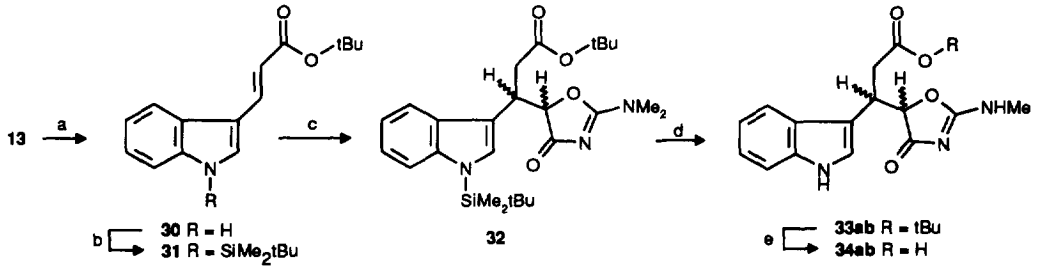
The *gem*dimethyl compound **20** could not be synthesised by the method of **Scheme 3**. Whereas the N-protected acetylindole **21**, prepared from acetyl indole **22** in an analogous manner to **14**, reacted successfully with methyl magnesium bromide to give the tertiary alcohol **23** in 62% overall yield, subsequent attempts to prepare the corresponding chloride proved fruitless. However, the fluoride **24** could be formed by reaction of **23** with diethylaminosulfurtrifluoride, and although the coupling of **24** with **8** proceeded in only low yield, the adduct **25** could be isolated and transaminated quantitatively to the desired product **20** by exposure to methylamine (**Scheme 4**). The desmethyl analogue **26** was prepared from gramine **27** without resort to N-protection. Selective quaternisation of the exocyclic nitrogen was achieved by treatment with iodomethane in ethanol[7] and this product **28** reacted directly with the oxazolinone lithium enolate **8** to give the displacement product **29**, in 10% overall yield. As before, transamination with methylamine afforded **26** in excellent yield.



Scheme 4 a) NaH, THF, $PhCH_2OCOCl$; b) MeMgBr, THF; c) DAST, CH_2Cl_2; d) **8**, THF; e) NH_2Me, sealed tube; f) MeI, EtOH

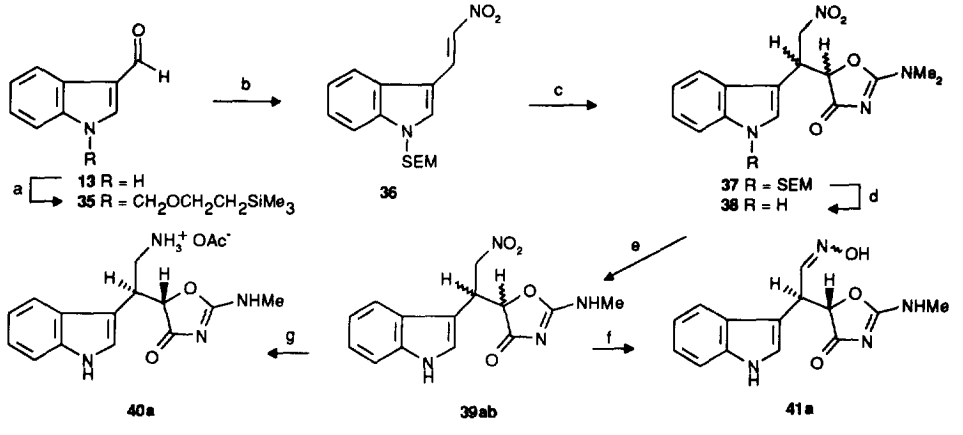
The introduction of an ester side chain required a different strategy (**Scheme 5**). The *tert*butyl ester of carboxymethylenetriphenylphosphorane condensed with indole-3-carboxaldehyde **13** to give the *trans*-alkene

30 in 92% yield. The indole nitrogen was deprotonated with sodium hydride and protected by silylation to give **31**. This compound reacted with the oxazole lithium enolate **8** to give the two diastereomeric Michael adducts **32** in 20% overall yield. These reacted with methylamine and caesium fluoride to afford the indolmycin analogues **33ab** in 85% yield. The corresponding acids **34ab** were formed in 80% yield by subsequently removing the *tert*butyl groups of **33ab** with trifluoroacetic acid in the presence of ethane-1,2-dithiol.



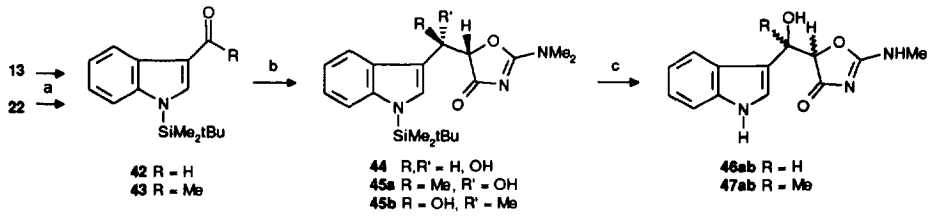
Scheme 5 a) $Ph_3P{=}CHCO_2tBu$, 1,4-dioxane; b) NaH, THF, $ClSiMe_2tBu$; c) **8**, THF; d) NH_2Me, sealed tube then CsF, MeOH, wet SiO_2; e) TFA, $HSCH_2CH_2SH$

Analogues containing nitrogen in the side chain were formed by a similar procedure (**Scheme 6**). In this case it was preferable to protect the indole nitrogen prior to olefination. The reaction of **13** with SEM chloride gave **35** which condensed with nitromethane in the presence of an ammonium acetate buffer to permit the construction of **36**. This αβ-unsaturated compound reacted with enolate **8** to give the diastereomeric adducts **37** in 71% overall yield. Removal of the SEM group to form **38** was effected in 65% yield using acidified lithium tetrafluoroborate.



Scheme 6 a) NaH, THF then $ClCH_2OCH_2CH_2OSiMe_3$; b) CH_3NO_2, NH_4^+ ^-OAc; c) **8**, THF; d) $LiBF_4$, $HSCH_2CH_2SH$, TFA; e) NH_2Me sealed tube; f) Bu_3SnH, AIBN, Toluene; g) H_2, PtO_2, HOAc

The separated diastereomers of **38** were transaminated as before to afford **39ab** in 50% yield. Reduction of the nitro group of **39a** (the better inhibitor, *vide infra*) using prehydrogenated platinum oxide in acetic acid afforded the amino analogue **40a**. By contrast, radical reduction of **39a** with tributyltin hydride gave the oxime **41a**, also in good yield.



Scheme 7 a) NaH, THF then $Me_2tBuSiCl$; b) **8**, THF; c) NH_2Me, sealed tube then CsF, wet SiO_2, MeOH

Finally, two groups of compounds were prepared by the direct reaction of the oxazole lithium enolate **8** with N-silylated indole-3-carboxaldehyde **42** and 3-acetylindole **43**. Despite a potentially favourable reverse aldol reaction, both diastereomers of **44** and **45** could be isolated chromatographically in 60-65% yield. The structure of **45a** was determined by X-ray crystallography. Transamination and removal of the silyl protection gave the hydroxyl analogues **46ab** and **47ab** in 45% and 35% yield respectively over the two steps.

R_1	R_2	R_3	Structure	MIC (μg/ml)	I_{50} (ng/ml)
Me	H	H	**1**	0.125	16 (mean)
H	Me	H	**10**[6]	8	500
Et	H	H	**11a**	64	250
H	Et	H	**11b**	>512	22000
Me	H	Me	**12a**	64	1200
H	Me	Me	**12b**	>512	>100,000
Me	Me	H	**20**	32	580
H	H	H	**26**	64	1550
$CH_2CO_2{}^tBu$	H	H	**33a**	>512	>100,000
H	$CH_2CO_2{}^tBu$	H	**33b**	>512	>100,000
CH_2CO_2H	H	H	**34a**	>512	>100,000
H	CH_2CO_2H	H	**34b**	>512	>100,000
CH_2NO_2	H	H	**39a**	64	2300
H	CH_2NO_2	H	**39b**	>64	>100,000
CH_2NH_2	H	H	**40a**	>512	>100,000
CHNOH, H	H	H	**41a**	>512	>100,000
H	OH	H	**46a**	>64	44000
HO	H	H	**46b**	>64	4000
Me	OH	H	**47a**	0.25	46
HO	Me	H	**47b**	>64	>100,000

Standard Deviation for I_{50} of racemic indolmycin (run as a standard) = 18.
Estimate of accuracy for individual I_{50} values = +/- 20% based upon curve fitting.

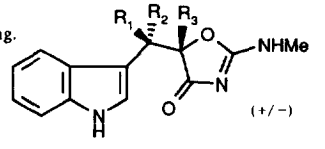
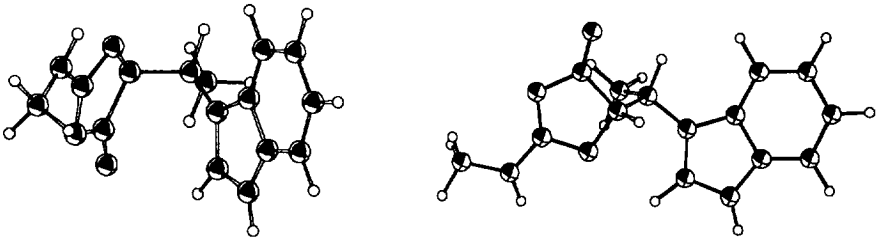


Table 1: Comparison of minimum inhibitory concentration (MIC) and I_{50} values for 5-and 5'-substituted analogues.

The MIC values against *Staphylococcus aureus* Oxford and the I_{50} values against the isolated enzyme from this bacterium are indicated in **Table 1**.[9] The relative stereochemistry of these indolmycin analogues was provisionally assigned by relating their physical and spectroscopic properties to indolmycin or to the hydroxyl analogue **45a**, for which the relative stereochemistries are known. In pairs of diastereomers, the better inhibitors were chromatographically the less polar isomers, and possessed the relative stereochemistry of indolmycin.[10] The ~33 fold variation in the ratio MIC / I_{50} may reflect differences in penetration associated with either altered lipophilicity, or affinity for the tryptophan active transport mechanism (*vide supra*). The lack of activity of the

carboxylic acid and amino analogues **34ab** and **40** militates against the theory that the methyl group represents the amino acid backbone. The ineffective nature of the hydroxy compounds **46ab**, those with groups significantly larger than methyl **33ab**, **39ab**, or compounds with adjacent steric bulk **12ab**, indicates that the methyl group binds in a small, essentially hydrophobic binding pocket. Equally, the weak inhibitory properties of the desmethyl compound **26** support the hypothesis that the methyl group does stabilise a particular conformation of the indole and oxazole moieties. This analysis is borne out in molecular modelling studies.

A comparison of the Ramachandran plots of calculated *CHARMm* energy[11] for rotation about the two bonds connecting the oxazole and indole moieties, supports an indolmycin conformation in which the hydrogen on C-5' lies broadly in the plane of the indole, pointing towards the benzenoid ring. The good inhibitor **47a** adopts very similar dihedral angles to **1** while the dimethyl analogue **20**, also disubstituted on C-5' but a much poorer inhibitor, does not. The conformation of the oxazole group relative to the bridging carbon is less clear; the two most energetically favoured minima for **1** are shown below.[11] Compounds such as **10**, **11b** or **12b**, for which these conformations represent a relatively high energy state, are poor inhibitors. The diagrams show the *E*-2-methylaminooxazolinone but the conclusions are equally valid for the *Z* geometry.



In order to determine whether one of these structures represents the binding conformation of indolmycin, our results have encouraged us to synthesise constrained analogues of indolmycin in which the C-4 position of the indole is fused onto the bridging carbon atom. Preliminary results of this study have been reported elsewhere.[12] The effects of independently modifying the aromatic and oxazole groups are currently under investigation.

References and Notes

1. Von der Haar, F.; Gabius, H-J.; Cramer, F. *Angewandte Chemie, Intl. Eng. Ed.*, **1981**, *20(3)*, 217-302.
2. "The Chemistry of Pseudomonic Acid Part 14", Broom, N. J. P.; Elder, J. S.;. Hannan P. C. T; O'Hanlon, P. J.; Pons, J. E.; Walker, G.; Wilson J.; Woodall, P. *Journal of Antibiotics*, **1995**, *48(11)*, 1336-1344 and references cited therein.
3. Rao, K. V. *Antibiot. Chemother.*, **1960**, *10*, 312. Werner, R. G. *Antimicrob. Ag. and Chemotherapy*, **1980**, *18*, 858.
4. Dirlam, J. P. U.S. Patent no. 4,584,385, 1986, Pfizer. Dirlam, J. P.; Clark, D. A.; Hecker, S. J. *J. Org. Chem.*, **1986**, *51*, 4920.
5. Werner, R. G.; Demain, A. L. *J. Antibiot.*, **1981**, *34(5)*, 551.
6. Werner, R. G.; Thorpe, L. F.; Reuter, W.; Nierhaus, K. H. *Eur. J. Biochem.*, **1976**, *68*, 1.
7. Snyder, H. R.; Smith, C. W.; Stewart, J. M. *J. Am. Chem. Soc.*, **1944**, *66*, 200.
8. This sample of isoindolmycin **10** was contaminated with ~3% indolmycin **1**, responsible for the observed inhibitory activity. **10** is reported to have no measurable antibacterial activity by Schach von Wittenau, M.; Els, H. *J. Am. Chem. Soc.*, **1963**, *85*, 3425.
9. The authors wish to acknowledge the contributions made by members of the Analytical Sciences and Microbial Cell Sciences Departments of SmithKline Beecham Pharmaceuticals, Brockham Park, in testing these compounds. I_{50} assays were performed using a variation of the method described by Durekovic, A.; Flossdorf, J; Kula, M-R. *Eur. J. Biochem.*, **1973**, *36*, 528.
10. Assignment of relative stereochemistry follows comparison of the 1H n.m.r. spectra of the 2-dimethylaminooxazole precursors.
11. Calculations performed using the *QUANTA* program from MSI; the diagrams show AM1 minimised representations.
12. Witty, D. R.; Walker, G.; Bateson, J. H.; O'Hanlon, P. J.; Eggleston, D. S.; Haltiwanger, R. C. *Tetrahedron Lett.*, **1996** *37(17)*, 3067.

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